INHIBITION OF TUMOR GROWTH BY MACROPHAGE INTERVENTION

FIELD OF THE INVENTION

The present invention relates to the field of cancer therapy.

BACKGROUND THE INVENTION

Angiogenic cellular proliferative diseases associated with the presence of solid tumors, particularly cancer, pose a significant health problem for the world population. In such diseases, cells grow and proliferate abnormally, in many cases in a manner that is ultimately lethal to their host. A wide variety of different treatment modalities have been developed in response to the desire to either halt or reverse the progression of cellular proliferative diseases. Such treatment modalities include surgery, radiation therapy and chemotherapy.

Tumors cannot grow beyond a certain size without angiogenesis, *i.e.*, once malignant transformation has occurred, every increase in tumor cell population must be preceded by formation of new capillaries that converge upon the tumor. Thus, the tumor growth profile is characterized by two phases: (a) a pre-vascular phase and (b) a vascular phase. A potentially malignant tumor can stay in a "dormant" stage for a number of years, with no invasion of surrounding tissue. The critical event that converts a dormant tumor into a rapidly growing malignancy is the switch to the angiogenic phenotype which demarcates the two phases in the development of the tumor - the pre-vascular phase and the vascular phase.

Angiogenesis, a fundamental process by which new blood vessels are formed, is highly regulated in adult mammals under normal physiological conditions. Angiogenesis requires the interaction of multiple cell types including endothelial cells, fibroblasts and macrophages, each signalling adjacent cells through soluble factors via cell receptors. In physiological angiogenesis, *e.g.*, pregnancy, wound

healing, negative inhibitory influences ultimately stop the process of neovascularization. However, in pathophysiological conditions like cancer, chronic inflammations and diabetic retinopathy the inhibitory controls fail and unrestrained angiogenesis occurs. Furthermore, with cancer, much experimental evidence exists showing that neovascularization is necessary for successful tumor growth and metastases.

Although a variety of different modalities are known for the treatment of cellular proliferative diseases such as cancer, to date there continues to be types of cellular proliferative diseases for which there are no cures, and treatment by currently available methods results at best in slower growth and relief from pain.

Accordingly, there is continued interest in the development of additional treatment modalities to further expand the armamentarium available to those health care practitioners who treat individuals suffering from cellular proliferative diseases, such as cancer.

SUMMARY OF THE INVENTION

Invention methods are focused on inhibition of angiogenesis in a variety of cell populations. Inhibition of angiogenesis is accomplished by inhibiting a host cell angiogenic effect. Most typically the host cell will be a macrophage which potentiates the so-called "macrophage angiogenic effect." More specifically, there are provided methods for inhibiting tumor growth in a mammalian host. In accordance with invention methods, the "macrophage angiogenic effect" is inhibited in at least the region of the tumor. The "macrophage angiogenic effect" may be inhibited, in at least the region of the tumor, by providing for an environment free of activated macrophages and/or blocking the effects of factor(s) derived from activated macrophages which are necessary for angiogenic factors to cause angiogenesis in tumors, resulting in inhibition of tumor growth beyond the pre-vascular phase. The environment free of activated macrophages may be provided by depleting at least the

region of the tumor of activated macrophages and/or preventing macrophage activation.

Invention methods find use in the treatment of wound healing and cellular proliferative diseases, *e.g.*, diabetic retinopathy, cancer, particularly cancers associated with the presence of solid tumors, and the like. Invention methods may be used in conjunction with one or more additional treatment modalities, such as surgery, radiation therapy, chemotherapy and the like.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 is a graphic depiction of data of tumor growth and angiogenic activity of three different human cancer cell lines (A673, DU 145, and MCF-7) implanted in dorsal skin fold chambers in NOD/LtSz-scid/scid mice. Open circles denote nude, and closed circles denote NOD/LtSz-scid/scid mice.

Fig. 2 is a graphic depiction of data comparing growth (upper left panel) and angiogenic activity (upper right panel) of the murine lung carcinoma cell line LLC implanted in dorsal skin fold chambers in NOD/LtSz-scid/scid and nude mice. The initial pre-vascular angiogenesis independent growth phase is indicated by dashed lines (first three days). The lower panel shows growth curves in terms of log (tumor volume) of LLC cells implanted s.c. in nude and NOD/LtSz-scid/scid mice.

Fig. 3 illustrates the effect of a blocking monoclonal anti-M-CSF (Macrophage colony stimulating factor) antibody (5A1) and a non-blocking (D24) antibody, on tumor growth and angiogenic activity growth pattern of the murine cell line (LLC) implanted in NOD/LtSz-scid/scid mice.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention there are provided methods for the inhibition of angiogenesis in a cell population in a mammal, said methods comprising inhibiting a host cell angiogenic effect in said mammal.

Invention methods find use in the treatment of cellular proliferative diseases characterized by increased angiogenic activity, *e.g.*, diabetic retinopathy, cancers, particularly those cancers characterized by the presence of solid tumors, and may be used in combination with one or more additional treatment modalities, including surgery, radiation therapy, chemotherapy, and the like.

In a particular aspect of the present invention, host cells are characterized as being subject to regulation by a macrophage colony stimulation factor (M-CSF). As used herein, macrophage colony stimulation factors include M-CSF-1 (also known as CSF-1). Host cells contemplated for treatment in accordance with invention methods include mast cells, fibroblasts, endothelial cells, macrophages, and the like.

Cell populations to which invention methods may be directed include hyperproliferative cells such as tumor cells, and other cell populations characterized by increased or unwanted angiogenic activity such as those present with diabetic retinopathy, psoriasis, and the like. In invention embodiments employing inhibition of the macrophage angiogenic effect, the macrophage angiogenic effect is inhibited in at least the region of the tumor, resulting in the inhibition of tumor growth beyond the pre-vascular phase.

In one embodiment of the present invention, the macrophage angiogenic effect is inhibited by at least one of: (a) administering to the host an agent that blocks the macrophage derived factor which acts with angiogenic factors to produce angiogenesis in tumors; or (b) providing for an environment free of activated

macrophages in at least the region of the tumor, where the macrophage free environment may be provided by depleting the region of activated macrophages and/or inhibiting macrophage activation.

Critical to one aspect of the present invention is the inhibition of the "macrophage angiogenic effect" in at least the region of the tumor such that growth of the tumor beyond the initial pre-vascular stage is at least slowed and usually substantially inhibited.

As used herein, the term "at least slowed" means that the rate of tumor growth beyond the pre-vascular stage is less than 20% and usually less than 5% of the natural rate of tumor growth; and the term "substantially inhibited" refers to the situation where the tumor grows at a rate that is less than 5%, usually less than 1%, and more usually is completely stopped so as to be 0% of the natural growth rate.

As used herein, "natural growth rate" means the rate of growth of a particular tumor in an analogous mammalian host environment that has not been subjected to any treatment modality, i.e., a control.

The term "macrophage angiogenic effect" as used throughout the specification and claims refers to the macrophage activity that is necessary for angiogenesis in tumors. Specifically, a "macrophage activity" is the factor released by activated macrophages which functions to produce angiogenesis in tumors in concert with angiogenic factors, such as VEGF, bFGF, Il-8, Angiostatin, Angiogenin, and the like.

In another embodiment of the present invention the macrophage angiogenic effect is inhibited in at least the region of the host in which the solid tumor is associated. As used herein, the terminology "at least in the region" includes both the localized region of the tumor and up to, and including, the entire host organism. Thus, through localized administration of agents which inhibit the macrophage

angiogenic effect, the inhibition can be limited to the local area of the tumor. As used herein, "local area" is defined as a particular organ, tissue or other naturally occurring physiological compartment or subunit of the anatomy of the host, or in a subportion thereof. Delivery of the inhibitory agent may be by any convenient means of localized delivery known to those of skill in the art. As will be appreciated by those of skill in the art, depending on the particular nature of the agent to be administered, localized delivery may be accomplished simply by administering the agent directly to the site of the tumor. In one embodiment of the present invention, the agent being administered may be administered in a depot composition which serves to retain the agent at the site of administration.

In another embodiment of the present invention, the macrophage angiogenic effect may be inhibited in the entire host organism, *e.g.*, by systemic administration to the mammalian host having the tumor. Any convenient means of systemic administration may be employed, *e.g.*, orally, topically, intravenously, parenterally, nasally, *e.g.*, in the form of an aerosol, and the like. As will be appreciated by those of skill in the art, the particular route of administration employed will generally be chosen with respect to the particular nature of inhibiting agent that is used.

In one embodiment of the present invention, the active agents, *e.g.*, macrophage depleters, M-CSF blockers, and the like, will generally be administered in a physiologically acceptable vehicle. The nature of the vehicle will generally depend on the nature of the agent being administered, *e.g.*, whether the agent is hydrophobic or hydrophilic, as well as the route of administration, *e.g.*, local or systemic, where suitable vehicles for various manners of administration are known in the art. For example, with hydrophobic agents, one may wish to employ a physiologically acceptable vehicle which promotes the solubility of such agents. Furthermore, as described above, where localized administration is desired, agents may be selectively targeted to the desired cell population. Delivery vehicles that act as depots or reservoirs for the agent may be employed; such vehicles may include

proteinaceous matrices, e.g., collagen, hydroxyapatite, and the like. For nasal delivery, the methods and devices described in U.S. Patent No. 5,458,135, the disclosure of which is incorporated herein by reference in its entirety, may be employed.

Compositions contemplated for use in the practice of the present invention can be used in the form of a solid, a solution, an emulsion, a dispersion, a micelle, a liposome, and the like, wherein the resulting composition contains one or more of the active compounds contemplated for use herein, as active ingredients thereof, in admixture with an organic or inorganic carrier or excipient suitable for nasal, enteral or parenteral applications. The active ingredients may be compounded, for example, with the usual non-toxic, pharmaceutically or physiologically acceptable carriers for tablets, pellets, capsules, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, suppositories, solutions, emulsions, suspensions, hard or soft capsules, caplets or syrups or elixirs and any other form suitable for use. The carriers that can be used include glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides, dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form. In addition auxiliary, stabilizing, thickening and coloring agents may be used. The active compounds contemplated for use herein are included in the pharmaceutical composition in an amount sufficient to produce the desired effect upon the target process, condition or disease.

In addition, such compositions may contain one or more agents selected from flavoring agents (such as peppermint, oil of wintergreen or cherry), coloring agents, preserving agents, and the like, in order to provide pharmaceutically elegant and palatable preparations. Tablets containing the active ingredients in admixture with non-toxic pharmaceutically acceptable excipients may also be manufactured by known methods. The excipients used may be, for example, (1) inert diluents, such as calcium carbonate, lactose, calcium phosphate, sodium phosphate, and the like; (2) granulating

and disintegrating agents, such as corn starch, potato starch, alginic acid, and the like; (3) binding agents, such as gum tragacanth, corn starch, gelatin, acacia, and the like; and (4) lubricating agents, such as magnesium stearate, stearic acid, talc, and the like. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract, thereby providing sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. The tablets may also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874, incorporated herein by this reference, to form osmotic therapeutic tablets for controlled release.

When formulations for oral use are in the form of hard gelatin capsules, the active ingredients may be mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate, kaolin, or the like. They may also be in the form of soft gelatin capsules wherein the active ingredients are mixed with water or an oil medium, for example, peanut oil, liquid paraffin, olive oil and the like.

Formulations may also be in the form of a sterile injectable suspension. Such a suspension may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,4-butanediol. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides, fatty acids (including oleic acid), naturally occurring vegetable oils like sesame oil, coconut oil, peanut oil, cottonseed oil, etc., or synthetic fatty vehicles like ethyl oleate or the like. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

Formulations contemplated for use in the practice of the present invention may also be administered in the form of suppositories for rectal administration of the active

ingredients. These compositions may be prepared by mixing the active ingredients with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters of polyethylene glycols (which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the active ingredients), and the like.

In addition, sustained release systems, including semi-permeable polymer matrices in the form of shaped articles (e.g., films or microcapsules) can also be used for the administration of the active compound employed herein.

In yet another embodiment of the present invention, the macrophage angiogenic effect is inhibited by depleting at least the region of the host containing the solid tumor of any activated macrophages, preferably providing, for the target cell population, an environment that is substantially free of activated macrophages. The depletion may be accomplished by administering an agent which is selectively cytotoxic for activated macrophages, where such agents include, bone resorption inhibitors, *e.g.*, diphosphonates, *e.g.*, etidronate disodium (etidronate) and (3-amino-1-hydroxy-propylidene)-1, 1-diphosphonate (ADP, pamidronate); and the like in a suitable delivery vehicle, e.g., a liposome capsule agent. Means of selectively depleting activated macrophages are known in the art and include those means described in: Berg *et al.*, J. Appl. Physiol. (1993) 74:2812-2819; Biewenga *et al.*, Cell Tissue Res. (1995) 280:189-196; Buiting and Van Rooijen, J. Drug Target (1994) 2:357-362; Camilleri *et al.*, Inflamm. Res. (1995) 44: 152-157; Gayton *et al.*, J. Endocrinol. (1996) 150:57-6; Gayton *et al*, J. Reprod. Fertil. (1994) 101:175-182; Quian *et al.*, J. Immunol. (1994) 152:5000-5008; Rezzani et al., Arch. Histol. Cytol. (1995) 58:427-433; Van Rooijen & Sanders, Hepatology (1996) 23:1239-1243; Van Rooijen & Sanders, J. Immunol. Methods (1994) 174:1-2, 83-93, the disclosures of each of which are incorporated herein by reference in their entirety.

According to another embodiment of the present invention, there are provided means of inhibiting the macrophage angiogenic effect on a cell population by

providing to the cell population an environment that is substantially free of activated macrophages. This may be achieved by a variety of methods, including inhibiting the activation of macrophages and/or inhibiting monocyte recruitment to said environment.

In still another embodiment of the present invention, alternatively or in combination with methods of selectively depleting activated macrophages in the region of a tumor, activation of macrophages in the tumor region is prevented in at least the region of the tumor by administering to the host a macrophage activation inhibition agent, where such agents include agents which block macrophage activation, inhibit recruitment of macrophages to the site of the tumor and or physically sequester M-CSF. Agents which block macrophage activation include: M-CSF receptor antagonists, M-CSF blocking agents, *e.g.,* small molecule mimetics (i.e., small organic or inorganic molecules, small peptides and the like), anti-M-CSF receptor antibodies, M-CSF antagonists, anti M-CSF antibodies, monoclonal anti-M-CSF antibodies, including those described in U.S. Pat. No. 4,504,586 the disclosure of which is incorporated herein by reference in its entirety, those secreted by the cell lines deposited and assigned ATCC Nos. HB-8208 and HB-8207; those described in J. Exp. Med. (1991) 173:1227-1234; those described in U.S. Pat. No 5,491,065, the disclosure of which is incorporated herein by reference in its entirety, antibody 5A1 as described in Balakrishna et al, J. Immunol (1988) 141:483-488, and the like. Agents that prevent macrophage activation by inhibiting monocyte recruitment to the area of the tumor include anti-CD18 monoclonal antibodies, as secreted by the cell lines IB4 and 2E6 having ATCC Deposit numbers of HB 10164 and HB 226, respectively, anti-CD29, anti-CD24, monocyte rolling preventing agents (e.g., anti-P and anti-E selectin antibodies), and the like.

In another aspect of the present invention, there are provided methods for the inhibition of angiogenesis in a cell population in a mammal, said methods comprising inhibiting a host cell angiogenic effect in said mammal, wherein inhibiting said host

cell angiogenic effect is accomplished by reducing the activity of a host cell-effecting factor, wherein said host cell-effecting factor, acting alone or in combination with one or more angiogenic factors, potentiates the macrophage angiogenic effect. In accordance with another aspect of the present invention, it has been discovered that in the absence of activated macrophages, angiogenic factors such as VEGF, bFGF, Il-8, angiostatin, angiogenin, and the like, which are secreted by tumor cells, are not able to induce angiogenesis and vascularization of the tumor. Thus, there is an activated macrophage-derived factor or factors that work in concert with known angiogenic factors, *e.g.,* VEGF, bFGF, Il-8, angiostatin, angiogenin, and the like, to result in vascularization of the tumor. In a particular aspect of the present invention, there are provided methods for the inhibition of angiogenesis in a cell population in a mammal, said methods comprising administration of one or more of these agents which block the activity of activated macrophage-derived factors that work in concert with angiogenic factors to promote angiogenesis. Host cell effecting factors contemplated for use with these embodiments of the present invention include macrophage colony stimulation factors such as M-CSF-1, and the like.

In still another embodiment of the present invention, there are provided methods for the inhibition of angiogenesis in a cell population in a mammal, said method comprising inhibiting a host cell angiogenic effect by inhibiting M-CSF gene expression. As will be appreciated by those of skill in the art, there are numerous methods available to inhibit the expression of a gene, all of which are contemplated for use in accordance with the present invention methods. In a particular aspect of the present invention, inhibition of M-CSF gene expression is accomplished by binding one or more anti-sense oligonucleotides to a sense-strand of DNA encoding said M-CSF gene (See, e.g., SEQ ID NO:1 for the sequence of human CSF-1 mRNA). Exemplary antisense oligonucleotides include GTCATACGGGGCAGCTGGCT (SEQ ID NO:2) which is complementary to nucleotides 91-110 of SEQ ID NO:1. In another aspect of the present invention, inhibiting M-CSF gene expression is accomplished by infecting a M-CSF -producing cell with a retrovirus or adenovirus.

As will be readily understood by those of skill in the art, the dosage of agent administered will vary widely depending on particular nature of the agent, the type of cellular proliferative disease, the nature of the host, and the like, where the dosage to be administered may be determined empirically by those of skill in the art.

Methods according to the present invention find use in the treatment of mammalian hosts suffering from cellular proliferative diseases, particularly cellular proliferative diseases characterized by increased angiogenic activity and or associated with the presence of solid masses or tumors. Cellular proliferative diseases include neoplasias, hyperplasias, psoriasis, diabetic retinopathy, and the like. A variety of mammalian hosts may be treated according to the subject invention. Such hosts include rare or valuable mammals, pets and livestock, humans, and the like.

As discussed above, the subject methods result in at least a slowing or retardation in the rate of the growth of a solid tumor beyond the pre-vascular stage, where the subject methods may result in substantial and even complete inhibition of the growth of the tumor beyond the pre-vascular stage.

Invention methods may be used in combination with additional treatment modalities, including surgery, radiation therapy and chemotherapy. Methods of surgery for both biopsy and reduction or elimination of tumor mass are known to those of skill in the art. Radiation therapy is also known to those of skill in the art and includes electromagnetic radiation, *e.g.*, high frequency x-rays, and subatomic particle radiation, *e.g.*, alpha particles, beta particles, neutrons, protons, mesons, and heavy ions. Finally, a variety of chemotherapeutic agents and methods for their use in cancer therapy are known and include: alkylating agents, *e.g.*, Mechlorethamine hydrochloride (Nitrogen Mustard, Mustargen, HN2), Cyclophosphamide (Cytovan, Endoxana), Ifosfamide (IFEX), Chlorambucil (Leukeran), Melphalan (Phenylalanine Mustard, L-sarcolysin, Alkeran, LPAM), Busulfan (Myleran), Thiotepa (Triethylenethiophosphoramide), Carmustine (BiCNU, BCNU), Lomustine (CeeNU,

CCNU), Streptozocin (Zanosar), and the like; plant alkaloids, *e.g.*, Vincristine (Oncovin), Vinblastine (Velban, Velbe), Paclitaxel (Taxol), and the like; antimetabolites, *e.g.*, Methotrexate (MTX), Mercaptopurine (Purinethol, 6-MP), Thioguanine (6-TG), Fluorouracil (5-FU), Cytarabine (Cytosar-U, Ara-C), Azacitidine (Mylosar, 5-AZA), and the like; antibiotics, *e.g.*, Dactinomycin (Actinomycin D Cosmegen), Doxorubicin (Adriamycin), Daunorubicin (duanomycin, Cerubidine), Idarubicin (Idamycin), BJeomycin (Blenoxane), Picarnycin (Mithramycin, Mithracin), Mitomycin (Mutarnycin), and the like, and other anticellular proliferative agents, *e.g.*, Hydroxyurea (Hydrea), Procarbazine (Mutalane), Dacarbazine (DTIC-Dome), Cisplatin (Platinol) Carboplatin (Paraplatin), Asparaginase (Elspar) Etoposide (VePesid, VP-16213), Amsarcrine (AMSA, *m*-AMSA), Mitotane (Lysodren), Mitoxantrone (Novatrone), and the like.

In using the subject methods in combination with one or more of the above reviewed conventional treatment modalities, the timing of the different modalities may be controlled so as to obtain optimum results with regard to substantial inhibition of progression and preferably complete remission of the cellular proliferative disease.

The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLES

Example 1

The ability of tumor spheroids of three different human tumor cell lines to grow following implantation in the dorsal skin fold chambers of NOD/LtSz-scid/scid and nude (nu/nu) mice was examined as follows. The NOD/LtSz-scid/scid mice were as described in Schultz *et al.*, J. Immunol. (1995) 154: 180-191 and the nude (nu/nu) mice were as described in Pelletier & Montplaisir, Methods Achiev. Exp. Pathol. (1975) 7:149-166. The three cell lines were the prostatic carcinoma cell line DU 145

(ATCC accession no. HTB-81), the breast carcinoma cell line MCF-7 (ATCC accession no. CRL-7910), and the rhabdomyosarcoma cell line A673 (ATCC accession no. HTB-22). The left panels in Figure 1 show growth (relative tumor area) of these tumor spheroids. The human tumors failed to grow significantly after the initial pre-vascular phase, *i.e.*,, angiogenesis independent phase, (three days) in NOD/LtSz-scid/scid mice, whereas tumors in nude mice continue to grow throughout the two week observation period. The right panels show angiogenic activity in arbitrary units according to below (5).0.0 = No Response, 0.5 = Dilated capillaries, 1.0 = Dilated and tortuous capillaries, 1.5 = Early budding, 2.0 = Extensive budding, 2.5 = E.xtensive budding starting to form vascular network, 3.0 = Early vascular networks with flow, 3.5 = Established but heterogenous vascular network. 4.0 = Established vascular network, 4.5 = High density vascular network 5.0 = Extremely high density vascular network.

These panels illustrate significant suppression of angiogenesis in NOD/LtSz-scid/scid mice for the different human tumor cell lines. Three days after implantation the MCF-7 cells in nude control mice, have induced early heterogenous vascular networks, whereas in the NOD/LtSz-scid/scid and nude anti-VEGF mice, there are dilated and some what tortuous muscle capillaries underneath the tumor spheroids. Seven days after implantation, vascular networks in tumors in nude control mice have become more homogenous and density has increased. In the chambers of NOD/LtSz-scid/scid and anti-VEGF mice at most budding of capillaries were found. During the last seven days, vascular density increases further in the nude control mice, whereas in the NOD/LtSz-scid/scid and anti-VEGF mice at most extensive budding can be seen. Similar suppression of angiogenesis and tumor growth was seen for human tumor cell lines implanted in nude mice after VEGF blockade. *See* Borgström, P. *et al.*, "Complete inhibition of angiogenesis and growth of microtumors by anti-vascular endothelial growth factor neutralizing antibody: Novel concepts of angiostatic therapy from intravital video microscopy," Cancer Res. (1996) 56:4032-4039. Note the significant suppression of growth and angiogenic activity, which is

very similar to the growth characteristics obtained after VEGF blockade in nude mice. The initial pre-vascular angiogenesis independent growth phase is indicated by dashed lines (first three days).

Example 2

The murine Lewis Lung cell carcinoma cell line (LLC) (ATCC NO. CRL-1642 was implanted in NOD/LtSz-scid/scid and nude (nu/nu) mice).

Figure 2(a) shows growth curves of these tumor spheroids (relative tumor area), and illustrates that LLC cells during the initial pre-vascular phase (three days) grew to the same extent in both types of mice. During the subsequent 4 days, tumors in nude mice continued to grow, whereas in the NOD/LtSz-scid/scid mice, there was still no significant growth. During the last seven days, tumors in nude mice continued to grow rapidly, whereas in the NOD/LtSz-scid/scid mice, growth was finally initiated. Panel b depicts angiogenic activity from these experiments. The extremely angiogenic LLC cells (38) induced early heterogenous vascular networks in nude mice already during the first three days NOD/LtSz-scid/scid mice displayed at most early budding during this phase. Seven days after implantation extremely high density vascular networks were found in nude mice, whereas in the NOD/LtSz-scid/scid mice, vascular activity was limited to at most low density heterogenous vascular networks. At the end of the two week observation period, high density vascular networks were found in both types of mice.

Example 3

Male mice (6-8 weeks) were injected subcutaneously with 1×10^6 cells in the dorsal area in a volume of 0.1 ml. Tumor volume (V) of subcutaneous tumors were calculated according to: $V = \pi/4 \times a \times b \times h$, where a, b, and h are long and short axes, and height of the tumor respectively. Tumor doubling time was calculated from the

slopes (β) of individual growth curves obtained by linear regression of ln (V) as a function of time. Doubling time (T^2) was calculated as: $T_2=\ln(2)/\beta$. Tumor doubling times were calculated and found to be 2.2±0.1, and 2.0±0.2 days in nude and NOD/LtSz-scid/scid mice respectively, which was not significantly different ($p=0.43$). Thus, growth rate of LLC tumors were equal in both strains of mice, however, with a 2.2±0.2 day delay in the onset of growth in the ND/LtSz-scid/scid mice.

Example 4

A subsequent series of experiments were designed to test if the delay in onset of angiogenesis and growth of LLC-tumors in NOD/LtSz-scid/scid mice could be ascribed to initial lack of and subsequent activation of residual tumor macrophages. In these experiments LLC tumor spheroids were implanted in dorsal skin fold chambers in NOD/LtSz-scid/scid mice and the ability of anti-M-CSF function blocking antibodies to inhibit onset of tumor angiogenesis was examined. Chambers were treated twice weekly either with a blocking (5A1) or non blocking (D24) monoclonal anti M-CSF antibody, as described in Balakrishna et al., J. Immunol. (1988) 141:483-488. In chambers treated with the control antibody LLC tumors grew but with a delay in the onset of growth. In mice treated with the neutralizing anti-M-CSF antibody, however, there was no further growth after the initial pre-vascular phase (first three days) (see Figure 3a)

In conclusion, the above results demonstrate that angiogenic activity of the human tumor cell lines DU145, A673 and MCF-7 was significantly inhibited when implanted in NOD/LtSz-scid/scid mice. The failure of these human tumor cell lines to support tumor angiogenesis is a result of the inability of the immature macrophages of NOD/LtSz-scid/scid mice to support tumor angiogenesis, as well as the inability of human tumor cell lines to activate these immature macrophages. The results demonstrating that anti-M-CSF mAb significantly inhibits tumor angiogenesis and suppresses growth of murine tumors beyond the initial pre-vascular phase in

NOD/LtSz-scid/scid mice further supports the conclusion that activated macrophages are prerequisites for tumor angiogenesis.

The lack of angiogenic activity and the suppressed growth of human tumor spheroids implanted in dorsal skin fold chambers in NOD/LtSz-scid/scid mice are similar to those of the same cell lines implanted in nude mice, but treated with a function blocking anti-VEGF mAb, as described in Borgström *et al., supra*. Blockade of either of M-CSF or VEGF, results in complete inhibition of tumor angiogenesis, from induction of established vascular networks to at most extensive budding of capillaries, resulting in no further growth after the initial pre-vascular angiogenesis independent growth phase. From these results it is concluded that the role of macrophages in tumor angiogenesis is not to secrete substances that are directly angiogenic, but rather macrophages are prerequisites for growth factors, such as VEGF to exert its mitogenic effect on endothelial cells. Moreover, it is concluded from the above that depletion of residual tumor macrophages, and/or blockade of macrophage activation, maybe in combination with anti-VEGF treatment and conventional chemotherapy, is a means of treating cellular proliferative diseases associated with the presence of solid tumors.

The above results and discussion demonstrate that novel methods of inhibiting tumor growth are provided. The subject methods provide a much needed new modality for cancer therapy and may be used in conjunction with other cancer therapies, such as surgery, radiation therapy and chemotherapy.

All publications and patent applications cited in this specification are incorporated herein by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit and scope of that which is described and claimed.